What Animals Have Taught Humans About TB Transmission


Edward A. Nardell, MD
Brigham & Women’s Hospital, Division of Global Health Equity
Associate Professor, Harvard Medical School
& Harvard School of Public Health
Partners In Health, Boston, USA
No conflicts of interest
Spectrum of TB Transmission

Classical epidemiology – world, country, city

Outbreak Investigations, household, institutional, community

Mathematical modeling

Molecular epi: global, national, city, outbreaks

Closed Environments

Outbreaks

animal studies
Spectrum of TB Transmission

Classical epidemiology – world, country, city

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Closed Environments
Outbreaks
animal studies
Why Guinea Pigs?

- Guinea pigs
  - Good CMI
  - Moderate caseation
  - Disease usually heals except for cavity formation and bronchial spread
  - Hematogenous spread
  - Lungs destroyed by continuous caseous necrosis

- New Zealand White Rabbits
  - Moderate CMI
  - Much caseation
  - Disease heals except for cavity formation and bronchial spread
  - Hematogenous spread
  - Lungs destroyed by continuous caseous necrosis

- Modern adult humans
  - Moderate CMI
  - Much caseation
  - Disease heals except for cavity formation and bronchial spread
  - Hematogenous spread
  - Lungs destroyed by continuous caseous necrosis

- Rhesus monkeys
  - Moderate CMI
  - Much caseation
  - Disease heals except for cavity formation and bronchial spread
  - Hematogenous spread
  - Lungs destroyed by continuous caseous necrosis

- Isolated human populations
  - Moderate CMI
  - Much caseation
  - Disease heals except for cavity formation and bronchial spread
  - Hematogenous spread
  - Lungs destroyed by continuous caseous necrosis

- Infants
  - Moderate CMI
  - Much caseation
  - Disease heals except for cavity formation and bronchial spread
  - Hematogenous spread
  - Lungs destroyed by continuous caseous necrosis

- Immunocompromised humans
  - Moderate CMI
  - Much caseation
  - Disease heals except for cavity formation and bronchial spread
  - Hematogenous spread
  - Lungs destroyed by continuous caseous necrosis

From Arthur Dannenberg, Jr.  
Pathogenesis of Human Tuberculosis, p 264, fig 10.  
Insights from the Rabbit Model, ASM Press, 2006
TB Transmission:
Not well understood until 1960s

TB transmission, c. 1930
Wells/Riley Experimental TB Ward, 1956-62


Quantitative air sampling for TB
Lessons Learned in Riley/Wells Experiments

1. TB is an airborne infection
2. Patients vary greatly in infectiousness
3. Transmission factors include clinical presentation, strain differences and drug resistance, and most of all, the impact of effective treatment
4. Not all infections progress – also documented transient TB infection and reinfection
5. Concentrations in air are extremely low, but could explain rates of infection of student nurses in the pre-chemotherapy era
Riley: First Experiment – counted 71 infections
(135 infections if 6 – 13 mm reactions included)

<table>
<thead>
<tr>
<th>TST reaction</th>
<th>guinea pigs autopsied total</th>
<th>confirmed TB</th>
<th>% confirmed TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 5 mm</td>
<td>39/238</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6 – 13 mm - no necrosis</td>
<td>24/64</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>6 – 13 mm – necrosis</td>
<td>16/16</td>
<td>13</td>
<td>81</td>
</tr>
<tr>
<td>14+ mm – necrosis</td>
<td>55/55</td>
<td>52</td>
<td>95</td>
</tr>
</tbody>
</table>

GP survival after infection
(chamber aerosol exposure, 35 - 50 cfu dose)

The Airborne Infections Research (AIR) Facility
MDR Provincial Treatment, Center
Mpumalanga, RSA
AIR Collaborators:

- **MRC**
  - Matsie Mphahlele
  - Kobus Venter (& Willem Lubbe)
  - Martie van der Walt
  - Karin Weyer
  - Bernard Fourie
  - Lourens Robberts
  - Daan Goosen, Veterinarian

- **CSIR**
  - Sidney Parsons, engineer*

- **CDC**
  - Paul Jensen, engineer
  - Charles Wells
  - Paul Arguin

- **Mpumalanga Provence Health Dept & Specialized MDR TB Referral Center**
  - Nurses
  - Administration
  - Doctors
  - Patients

- **Harvard and Brigham & Women’s Hospital**
  - Edward Nardell, PI
  - *Melvin First*, engineer
  - Ashwin Dharmadhikari

- **Other**
  - Dave McMurray – Texas A & M
  - Ian Orme – Colorado State
  - Randall Basaraba – Colorado State
  - Paul Van Helden, Rob Warren, Elizabeth Streicher - Centre for Molecular and Cellular Biology, RSA

- **Funding**
  - USAID/CDC
  - MRC
  - Brigham & Women’s Hospital
  - Harvard CFAR (NIH) – two awards
  - NIOSH (NIH) RO1
  - NIH K23 (A. Dharmadhikari, PI)
AIR Pilot Study:
362 GPs exposed to 26 MDR-TB patients over 4 months
GP TST ≥ 6 mm

Observations from AIR Pilot Study

1. 53 guinea pigs reverted their skin test back to 0 mm.
2. No guinea pigs that reverted had signs of TB or pathology

33 guinea pigs reverted and had **reinfection**
Pathology – only 15% had pathological lesions
Human-like full range of outcomes - 362 gps

Exposure – no infection (91, 25%)

Exposure – infection (271, 75%)

Exposure – infection – reversion (53, 15%)

Exposure – infection – reversion - reinfection (33, 9%)

Exposure – infection – (reinfection) – disease (54, 15%)
Reported:
1) High rates of transmission from patients to guinea pigs
2) Transient TB infection
3) Reinfection
4) Limited disease progression
5) Spectrum of pathology not seen in chamber exposure studies
Experiments not designed or funded to study TB pathogenesis, but to test interventions.

**AIR, Experimental Plan**

Guinea Pig Air Sampling

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odd days</td>
<td>Even days</td>
</tr>
</tbody>
</table>

- UVGI or other intervention
- 3 patient rooms
- Plus common areas

**Intervention on/off on alternative days**
How Effective are Surgical Masks on Patients?

For patients

For health care workers

Respirators
How Effective Are Surgical Masks on Patients?

Approx 53% Effective

Dharmadhikari AS, et. al.
Am J Respir Crit Care Med.

NIOSH funded
Upper Room Germicidal UV

Ventilation ducts in patient rooms

Paddle fans assure good air mixing
## Results

<table>
<thead>
<tr>
<th></th>
<th>UV1 Intervention</th>
<th>UV1 Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>TST-1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TST-2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>TST-3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>TST-4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>0</td>
<td>9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>UV2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TST-1</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>TST-2</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>TST-3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>TOTAL*</td>
<td>15</td>
<td>48</td>
</tr>
</tbody>
</table>

*p<0.0005

**Combined** hazard ratio 4.9 (CI.95: 2.8, 8.6) or **about 80% effective.**

**Note:** 6 ACH (mechanical). Doubling to 12 EqACH would reduce risk by about 50%; Doubling to 24 EqACH would reduce risk by about 75%; so UVGI added about 24-6 (mechanical) = about **18 EqACH** to the AIR facility wards.
Novel approaches to UVGI

Innovative UVGI fixture alternatives: preliminary data:

Also testing LED UV but technology is expensive and early in development.

Figure 7: Total ceiling UVGI configuration. Artist’s rendering (left) and preliminary data (above)
Eggcrate Whole Upper Room UVGI

Lamp on wall rather than on eggcrate.

Fan

Eggcrate ceiling tiles

UV Lamp
Upper-room ultraviolet light and negative air ionization to prevent tuberculosis transmission

UV group
Ionizer group
No intervention group

UV vs. No intervention: Log rank 46 p<0.0001
Ionizers vs. No intervention: Log rank 20 p<0.0001
UVGI reduced TB: 72%
Ionisers reduced TB: 58%
TB transmission only from untreated patients – Peru

Escombe 2008 Plos Medicine; 5:e188

- 97 HIV+ pulmonary TB patients exposed 292 guinea pigs over 505 days
  - 66 cult +, 35 smear +
- 122/125 GP infections (98%) were due to 9 MDR patients
  - all inadequately or delayed treatment
    » 108/125 infections (86%) due to 1 MDR patient
  - 3 drug susceptible patients infected 1 guinea pig each
    » 2 had delayed treatment
    » 1 had treatment stopped
Riley Ward – 2\textsuperscript{nd} 2-year study
- included untreated patients

Relative infectivity of patients*:

- Susceptible TB
  - 61 Untreated (29 GPs) 100%
  - 29 Treated** (1 GP) 2%

- Drug-resistant TB
  - 6 Untreated (14 GPs) 28%
  - 11 Treated (6 GPs) 5%

*all smear positive patients, relative to the amount of time on the ward

**treatment started \textit{same day} they entered the ward.
Guinea Pig Transmission: South Africa

109 patients: smear +, cavitary, coughing, recently started on therapy

<table>
<thead>
<tr>
<th>Experiment</th>
<th># Patients/ Exp. Duration</th>
<th>% Guinea pigs infected (# exposed)</th>
<th>Patients # XDR (MGIT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pilot</td>
<td>26* / 4 mos</td>
<td>74% (360)</td>
<td>3/11</td>
</tr>
<tr>
<td>Exp 1</td>
<td>24 / 3 mos</td>
<td>10% (90)</td>
<td>5/10</td>
</tr>
<tr>
<td>Exp 2</td>
<td>15 / 2 mos</td>
<td>53% (90)</td>
<td>2/11</td>
</tr>
<tr>
<td>Exp 3</td>
<td>27 / 3 mos</td>
<td>1% (90)</td>
<td>0/21 0/27 (LPA)</td>
</tr>
<tr>
<td>Exp 4</td>
<td>17 / 3 mos</td>
<td>77% (90)</td>
<td>2/10</td>
</tr>
</tbody>
</table>

* 8 different spoligotypes, but only 2 transmitted to GPs – both XDR-associated
Unsuspected, untreated XDR TB
All other patients on effective treatment

MDR TB Ward
Potential for re-infection
TB Triage – Rapid DR Diagnosis

Smear status may not be critical if on effective treatment

Gene Xpert: TB, DS or MDR

Community based – *on effective treatment – responding*

Complications

Hospitalized patients *on effective treatment - responding*

Individual Isolation

Effect of treatment Unknown

Novel interventions

XDR by LPA

• **Find** TB cases - rapid diagnosis
  • Focus on rapid molecular diagnosis – Xpert TB
  • Sputum smear – can also be rapid, but more limited

• **Active** case finding
  • Focus on cough surveillance at all entrance points

• **Separate** safely and reduce exposure
  • Building design and engineering
  • Cough hygiene and triage

• **Treat** effectively, based on rapid DST
  • Focus on rapid molecular DST – Xpert TB
Cough generating airborne infectious respiratory droplets

Inhaled antibiotic: Nebulized kanamycin, or *Dry powder capreomycin*

or Colistin

Trachea and large airways

Antibiotic concentration 1000-100,000 X MIC

Microbes in epithelial fluid lining layer

Figure 1: Theoretical model and experimental approach to testing the inhaled antibiotics infection control hypothesis. (* indicates future planned studies)

Airborne infectious droplet nuclei

Measures of infection control potential:

- Mtb cultured in daily 12 hr sputum
- Mtb cultured in cough aerosol samples
- Infection rate of exposed guinea pigs*

Cough generating airborne infectious respiratory droplets

Antibiotic concentration 1000-100,000 X MIC

Rx
Where does BCG act?

*Can it prevent infection or reinfection?*

- **Human studies**
  - Many clinical trials – outcome: TB disease
    - No way to measure infection post-BCG
    - BCG about 50% effective in preventing serious complications of TB
    - Autopsy study: BCG prevents dissemination, not infection.
    - Party line: does not prevent infection

- **Animal studies**
  - Earlier work in GPs – does not prevent infection
  - Our natural GP model – wider range of outcomes that could be influenced by BCG
Our next study (BMGF-funded): Screening Vaccine Candidates

- In traditional chamber GP exposure studies, all animals get infected and all animals die
- Not human-like
- Unlikely that a vaccine can turn this around
Recent observations:  
**Based on IGRA Testing**  

- 2005: Turkey, among 979 child household contacts, presence of BCG scar associated with **0.6 odds** of infection (IGRA), compared to BCG vaccinated. *Lancet 2005; 366:1443* 

- 2009: UK school exposure. 29% BCG vaccinated had + IGRA compared to 47% unvaccinated. **(adj risk reduction, 74%)**  
  *Vaccine 2009; doi10.1016/j.vaccine* 

- 2010: UK nursery exposure. 42% of children and 60% of adults BCG vaccinated. **BCG 66% effective** in preventing infection.  
  *Thorax 2010; 65:1067-1071.*
BCG to Prevent TB Infection: *Immediate Need and wider Potential*

- Immediate use of BCG for adult US/other medical and humanitarian workers (students) in high MDR/XDR burden settings
  - ACET guidelines submitted for publication.
  - New observations: BCG prevents infection in children/adults
  - Clinical trial planned: IGRA conversion as end point

- Natural GP infection model to test the hypothesis that BCG prevents infection
  - Wide range of outcomes: prevent infection, prevent re-infection, prevent progression
  - If true for BCG, potential screen for newer vaccines
After Dannenberg:

Stage 1: Onset (First week)
- Innate microbicidal capacity:
  - Genetic priming
  - Non-specific activation
- Resident Alveolar Macrophage
- No infection

Stage 2: Logarithmic growth (Days 7 to 21)
- Blood-derived Immature monocytes
- Granuloma initiation
- Macrophage rupture
- $10^4$ bacilli
- DTH/CMI triggered

Stage 3: Immunologic control (After 3 weeks)
- Activated macrophages (CMI)
- Monocytes ruptured by Cytotoxic lymphocytes (DTH)
- Self-limited primary disease
- Solid caseation
- Granuloma maturation

Stage 4: Liquefaction (Lung cavitation)
- Post-primary Disease:
  - Endogenous reactivation
  - Exogenous reinfection
- (Progressive primary disease)

*macrophage activation accelerated by previously sensitized lymphocytes
Spectrum of Responses to Mtb Infection
(Doug Young. Trends in Microbiology 2009; 17:183-8)

- Infection eliminated without Priming ag specific T cells
- Infection eliminated in association with T cell priming
- *Infection controlled with some bacteria persisting in non-replicating form*
- Bacteria replication maintained at a subclinical level by immune response
- Clinical disease

- **Innate Immune**
  - Adaptive immune
  - *Quiescent Infection (LTBI)*
  - Active Infection
  - Disease

Bacterial Load or Dose (reinfection?)
AIR Proposal, v8.2
(320 animals)

All animals will be monitored and the Karnofsky scale (or similar criteria) will be used to identify animals for early termination.

Group 1, Naïve
N=140
*To correlate PPD w/cell-based assay

Group 2, BCG
N= 90
*BCG efficacy (exp. Group)

BCG (SSI) Week-10

Weeks 0 2 4 6 8 10 12 14 16

1mL bleed of 10 pre-selected animals from each of these 2 groups

ensure these 10x2 animals (same animals throughout) are not ones in the 8 and 12 week sacrifice sets.

Funded by BMGF, 2013

Bleeds at sacrifice will give enough cells for correlation studies. Cells will be stimulated (4 distinct conditions: negative control; positive control-PHA; ESAT6/CFP10; CFP10/MTP64). Supernatant frozen for future IGRA and cells used for qRT-PCR (IFNg +). Results of assays should correlate with skin test at each terminal timepoint.

Group 3, Naïve,
N=90
BCG efficacy (control group)

Week 16 is overloaded with sacrifices (although some will be brought down early when meet Karnofsky criteria). Will need to stagger week 16 sacrifices.
Class dismissed!